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Absence of nitroglycerin-induced heparin resistance in healthy volunteers

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A previously described nitroglycerin-induced heparin resistance could not be verified by in-vitro *experiments or in a randomized, double-blind, crossover trial in healthy volunteers. A clinically relevant attenuation of the anticoagulant effect of a heparin bolus (40 U.kg^{-1}) by a concomitant infusion of nitroglycerin (100 μg.min^{-1}) was absent. Activated partial thromboplastin time was not significantly different under nitroglycerin infusion as compared to placebo after heparin injection. Concentrations and activities of antithrombin III and heparin cofactor II remained unchanged during nitroglycerin infusion. An interaction of these two frequently combined drugs in patients with active thromboembolic disease or after a prolonged concomitant intravenous administration cannot be ruled out. Since this is of clinical importance, further studies must clarify a possible nitroglycerin-induced heparin resistance.*

Introduction

Anticoagulation with heparin is widely accepted in patients with acute coronary heart disease or after therapeutic interventions such as thrombolysis or coronary angioplasty[1–3]. The variable individual response to a standard dose of heparin is well known[4,5]. One possible explanation may be an interaction of heparin with co-administered drugs. In intensive care medicine, heparin is frequently used in combination with a continuous infusion of nitroglycerin. Previous reports have suggested an attenuation of the anticoagulant effect of heparin induced by concomitantly infused nitroglycerin preparations[6–8]. Based on our own observations of an increased need for heparin to maintain a therapeutic state of systemic anticoagulation in several patients with nitroglycerin infusions, this study examined a possible nitroglycerin-induced heparin resistance in vitro and with a randomized, double-blind, placebo-controlled crossover trial in healthy volunteers.

Materials and methods

The preparation of nitroglycerin used was a 0·5% solution of glycerol trinitrate in ethanol with polyoxyethylene-40-castoroil (2 mg.ml^{-1}) as emulgator (Trinitrosan, E. Merck, Darmstadt, Germany); the heparin used was sodium heparinate (Liquemin, F. Hoffmann-La Roche, Basel, Switzerland). Activated partial thromboplastin time (APTT, s) was performed according to the manufacturer (Aktin, Baxter Dade, Düdingen, Switzerland). Values in normal adult plasma range from 25–34 s, average 30 s. Heparin concentration (U.ml^{-1}) was measured as anti-Xa-activity[9] using an amidolytic assay (Coatest Heparin, Kabi Diagnostica, Mölndal, Sweden). For the amidolytic assay of antithrombin-III-activity (AT IIIa, % of normal) bovine thrombin and the chromogenic substrate chromozym TH (Boehringer, Mannheim, Germany) were used[9,10]. Immunoreactive antithrombin III (AT IIIi, % of normal) was measured by a quantitative electroimmunoassay[11] using a rabbit antiserum (Clottimmun-Antithrombin-III, Behring, Marburg, Germany). Heparin-cofactor II (HC II, % of normal) was measured as dermatan sulfate cofactor activity[12,13] after heparin neutralization by polybrene (Hexadimethrin bromide, Aldrich-Europe, Belgium) in the tris-EDTA-polybrene buffer (0·02 Tris, 0·15 M NaCl, 0·0075 M K_2EDTA, 0·1% PEG 6000, polybrene 2 μg.ml^{-1}, 0·02% NaH_3 pH 8·0) used for the 1:76 plasma predilution. In the test system dermatan sulfate (334 μg.ml^{-1} final concentration) specifically accelerated the heparin cofactor II/thrombin reaction[14]. The remaining free thrombin (human thrombin, Calbiochem, Lucerne, Switzerland) was measured amidolytically on chromozym TH. All amidolytic assays were run on a centrifugal analyser (Cobas, Roche). To minimize test-specific components of variability, all assays of heparin concentration, AT IIIa, AT IIIi, and HC II were performed from deep frozen plasma samples in one single run. In the range of values concerned, within-assay coefficients of variance for APTT, AT IIIa, AT IIIi, HC II, and heparin concentration are 3%, 4%, 6%, and 5%, respectively. The corresponding between-assay coefficients of variance are 5%, 7%, 9%, 12%, and 11%.

IN VITRO STUDY

Heparin was added to pooled, platelet-poor citrate plasma obtained from drug-free donors aiming at a final concentration of 0·3 U.ml^{-1}. Trinitrosan was added, resulting in nitroglycerin concentrations from 0·01 μg.ml^{-1} to 100 μg.ml^{-1}. In each sample coagulation

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Table 1 Coagulation parameters with increasing nitroglycerin concentrations

Heparin added (U.ml $^{-1}$)	NG added (μg.ml $^{-1}$)	APTT (s)	Heparin measured (U.ml $^{-1}$)	AT IIIa (%)	AT IIIi (%)	HC II (%)
0	0	30·1	0	—	—	—
0·3	H_2O	54·5	0·39	90·5	94	108
0·3	ethanol	54·7	0·38	90·5	94	115
0·3	0·01	53·5	0·34	98	90	110
0·3	0·1	54·3	0·33	97	90	117
0·3	0·5	56·0	0·33	97	87	93
0·3	1·0	56·9	0·34	90	89	98
0·3	5·0	56·8	0·31	97	90	104
0·3	10	52·2	0·35	96	90	97
0·3	50	53·7	0·39	100	99	103
0·3	100	53·1	0·29	103	93	100

APTT, activated partial thromboplastin time; AT IIIa: activity of antithrombin III; AT IIIi: concentration of antithrombin III measured immunologically; HC II, activity of heparin-cofactor II; NG: nitroglycerin concentration.

assays were performed in duplicate. Control values were obtained with aqua bidest and ethanol.

IN VITRO STUDY

Volunteers were male students (n = 7, mean age 28 ± 4 years, weight 77 ± 8 kg, platelet count 235 000 ± 54 000.ml^{-1}) with no history of coagulation disorders or thromboembolic disease. After obtaining baseline values of coagulation parameters, nitroglycerin (100 μg.min^{-1}) and saline 0·9%, respectively, were infused in a randomized, double-blind, crossover sequence. Fifteen minutes after the start of the infusion 40 U.kg^{-1} heparin were injected as a bolus. Five, 30, 60, 90, and 180 min after heparin injection, blood was drawn for coagulation tests. Infusion and blood sampling were performed through two different venous cannulas, one in each forearm. After return of coagulation parameters to baseline values the infusion was changed. After an equilibration period of 15 min the trial was repeated with the same volunteer.

The study was approved by the local review committee for human research, and informed, written consent was obtained from each volunteer.

Statistical evaluation was done by Student's t-test for paired values and two-way analysis of variance. A two-tailed *P*-value of $<0·05$ was considered statistically significant.

Results

In-vitro results are summarized in Table 1. Increasing concentrations of nitroglycerin did not alter the anticoagulant effect of heparin monitored by prolongation of APTT. Activity and concentration of AT III and activity of HC II remained unchanged. The nitroglycerin concentrations tested in vitro (0·01–100 μg.ml^{-1}) include and exceed plasma concentrations achieved by doses clinically used for intravenous therapy[15].

Average values of APTT after bolus injection of heparin in seven healthy volunteers under a continuous infusion of nitroglycerin (100 μg.min^{-1}) compared to placebo are shown in Fig. 1. There was no significant difference at any time APTT was measured. Baseline values of APTT were identical, and peak values after heparin were in the therapeutic range in all subjects. Heparin concentration was not affected by the nitroglycerin infusion (Fig. 2). At no time did the activities of antithrombin III and heparin-cofactor II change under nitroglycerin infusion (Fig. 3); the same was true for the concentration of antithrombin III measured immunologically (data not shown). No side effects from heparin bolus injection or nitroglycerin infusion were observed.

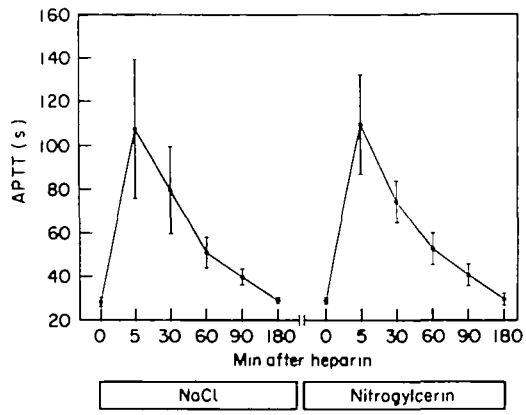

Figure 1 Activated partial thromboplastin time (APTT) in seconds under nitroglycerin infusion (100 μg.min^{-1}) compared to 0·9% saline in seven healthy volunteers after a bolus injection of heparin (40 U.kg^{-1}) (mean ± SD).

Discussion

The clinical importance of an interaction of heparin with infused nitroglycerin is evident. Frequent laboratory testing and dose adjustments of heparin in patients under nitroglycerin would be mandatory. The risk of bleeding complications after discontinuation of a nitroglycerin infusion would call for a dose reduction of heparin.

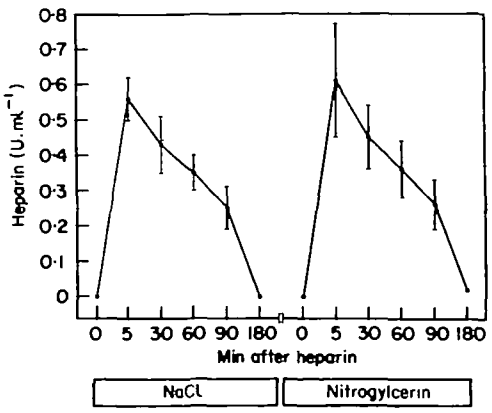

Figure 2 Concentration of heparin under nitroglycerin infusion (100 μg.min^{-1}) compared to 0·9% saline in seven healthy volunteers after a bolus injection of 40 U.kg^{-1} (mean ± SD).

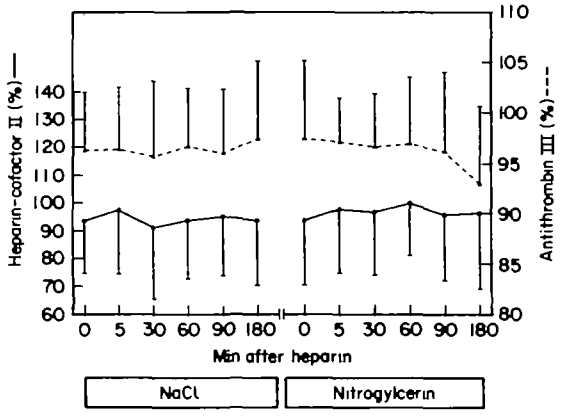

Figure 3 Activities of antithrombin III and heparin cofactor II under nitroglycerin infusion (100 μg.min^{-1}) compared to 0·9% saline in seven healthy volunteers after a bolus injection of heparin (40 U.kg^{-1}) (mean ± SD).

A nitroglycerin-induced 'heparin-resistance' may be explained by various mechanisms: (a) direct interaction on a molecular basis (b) formation of a nitroglycerin–plasmaprotein–complex inactivating the negatively charged heparin like protamin (c) depletion of coagulation inhibitors mediating the heparin effect (d) influence on heparin elimination or metabolism by nitroglycerin.

The results presented do not support the assumption of a clinically relevant nitroglycerin-induced heparin resistance. The in-vitro results rule out a major direct molecular interaction of the two drugs. The absence of an alteration in extent or duration of the anticoagulant effect of a heparin bolus monitored by APTT in a double-blind, placebo-controlled trial with crossover design strongly denies any important in-vivo interaction within the first 3 h after heparin injection. Heparin concentrations as well as the activities of the two major mediators of heparin effects remained unchanged, thus ruling out a substantial depletion of these factors by simultaneously infused nitroglycerin within the period investigated and with the drug preparations used.

These results contrast with previous reports which suggested a nitroglycerin-induced heparin resistance[6–8]. However, these observations described an attenuated heparin effect after several hours or even days of continuous, intravenous administration of both drugs[7,8]. A gradual change in metabolism of heparin or a functional depletion of AT III or HC II induced by nitroglycerin after longer periods of simultaneous infusion of both drugs remains possible. Our in-vivo experiment only investigated the immediate heparin effect. In the period of approximately 3 h with a measurable heparin effect after bolus injection, no interaction with nitroglycerin was observed.

A dose-response curve may be desirable to exclude definitely an in-vivo heparin–nitroglycerin interaction. A longer period of concomitant infusion of both drugs and higher doses of nitroglycerin might result in an attenuated heparin effect. However, nitroglycerin concentrations tested in the in-vitro experiment by far exceed plasma concentrations achieved by infusion rates used in clinical practice[15]. As a dose-dependent effect was absent in vitro, we chose to use only one common, therapeutic dosage in the in-vitro trial (100 μg.min^{-1}) and believe a clinically relevant effect was sufficiently ruled out. Furthermore, we were concerned about submitting volunteers to higher doses of intravenous nitroglycerin.

Another limitation of this study may be the fact that for ethical reasons we only tested healthy volunteers. Different results in patients with an active thromboembolic disease such as unstable angina cannot be ruled out. However, this seems unlikely as a recent investigation in patients undergoing coronary angioplasty also failed to show any significant nitroglycerin-induced heparin resistance[16].

The nitroglycerin preparations used in clinical practice may be of importance, too. In particular, the solvent propylene glycol has been incriminated as the substance inducing heparin resistance[6]. Since the preparation we used contains polyoxyethylene-40-castoroil as a nonionic emulsifying agent this could account for the differences in results. However, this seems unlikely, as both propylene-glycol-containing and propylene-glycol-free nitroglycerin preparations have been reported to attenuate heparin effects[7,8].

In conclusion, an immediate nitroglycerin-induced heparin resistance with a propylene–glycol-free nitroglycerin preparation was absent in vitro and in an in-vivo trial with healthy volunteers. An attenuation of the anticoagulant effect of heparin by nitroglycerin in patients with active thromboembolic disease or after a prolonged period of concomitant intravenous administration of both drugs cannot be ruled out. The clinical importance of such an interaction calls for further studies.

References

[1] Yusuf S, Sleight P, Held P, McMahon S. Routine management of acute myocardial infarction. Lessons from overviews of

recent randomized controlled trials. Circulation 1990; 82 (Suppl II): II-117–II-134.
[2] Laskey MAL, Deutsch E, Barnathan E, Laskey WK. Influence of heparin therapy on percutaneous transluminal coronary angioplasty outcome in unstable angina pectoris. Am J Cardiol 1990; 65: 1425–9.
[3] Chesebro JH, Badimon L, Fuster V. New approaches to treatment of myocardial infarction. Am J Cardiol 1990; 65: 12C–19C.
[4] Salzman EW, Deyhin D, Shapiro RM, Rosenberg R. Management of heparin therapy — A controlled prospective trial. N Engl J Med 1975; 292: 1046–50.
[5] Hirsh J, van Alken WG, Gallus AS, Dollery CT, Cade JF, Yung WL. Heparin kinetics in venous thrombosis and pulmonary embolism. Circulation 1976; 53: 691–5.
[6] Col J, Col-Debeys C, Lavanne-Pardonge E *et al.* Propylene glycol-induced heparin resistance during nitroglycerin infusion. Am Heart J 1985; 110: 171–3.
[7] Habbab MA, Haft JI. Heparin resistance induced by intravenous nitroglycerin. Arch Intern Med 1987; 147: 857–60.
[8] Pizzuli L, Nitsch J, Lüderitz B. Nitroglycerin inhibition of the heparin effect. Dtsch Med Wschr 1988; 113: 1837–40.
[9] Bounameaux H, Marbet GA, Lämmle B, Eichlisberger R, Duckert F. Monitoring of heparin treatment. Am J Clin Pathol 1980; 74: 68–74.
[10] Odegard OR. Evaluation of an amidolytic heparin cofactor method. Thromb Res 1975; 7: 351–60.
[11] Laurell CB. Quantitative estimation of proteins by electrophoresis in agarose gels containing antibodies. Anal Biochem 1966; 15: 45–52.
[12] Abildgaard U, Larsen ML. Assay of dermatan sulfate cofactor (heparin cofactor II) activity in human plasma. Thromb Res 1984; 35: 257–66.
[13] Marbet GA, Zbinden B, Duckert F Konstitutionelle Dysfunktion von Heparin-Kofaktor II mit venöser Thromboseneigung. Schweiz Med Wschr 1988; 118: 1586–8.
[14] Tollefsen FM, Pestka CA, Monafo WJ. Activation of heparin cofactor II by dermatan sulfate. J Biol Chem 1983; 258: 6713–6.
[15] Imhof PR, Sieber A, Hodler J *et al.* Plasma concentrations and haemodynamic effects of nitroglycerin during and after intravenous infusion in healthy volunteers. Eur J Clin Pharmacol 1982; 23. 99–106.
[16] Lepor NE, Amin DK, Berberian L, Shah PK. Does nitroglycerin induce heparin resistance? Clin Cardiol 1989; 12: 432–4.